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Surprising observations of *Polyergus rufescens* (Latreille, 1798) (Formicidae: Hymenoptera) in a repeated faunistic study of heathlands in the Belgian Province of Limburg

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Abstract

We report new records of the very rare and endangered European Amazon ant, *Polyergus rufescens* (Latreille, 1798) in the Province of Limburg, Belgium in the summer of 2020. During a large-scale invertebrate survey in large heathland patches in Zonhoven and Houthalen workers and queens were collected with pitfall traps and observed at the site. The importance of this finding and suggestions on heathland management in favour of this species as well as its survival in the north of Belgium are discussed.

Keywords: European Amazon ant, Formicidae, heathlands, new record

Samenvatting

We melden nieuwe waarnemingen van de zeer zeldzame en bedreigde Europese Amazonemier, *Polyergus rufescens* (Latreille, 1798) in de provincie Limburg, België tijdens de zomer van 2020. Gedurende een grootschalig invertebraten onderzoek in heidegebieden in Zonhoven en Houthalen, werden werksters en gynes ingezameld met bodemvallen en waargenomen op de site. Het belang van deze observaties alsook suggesties voor heidebeheer ten voordele van deze soort alsook het voortbestaan in het noorden van België worden besproken.

Résumé

Nous signalons de nouvelles observations de la très rare et menacée fourmi amazone d'Europe, *Polyergus rufescens* (Latreille, 1798) dans la province du Limbourg (Belgique), pendant l'été 2020. Durant un inventaire des invertébrés mené à large spectre dans les bruyères des communes de Zonhoven et Houthalen, tant des ouvrières que des reines ont été capturées au moyen de pièges et observées sur place. Cet article discute de l'importance de cette découverte ainsi que de la survie de cette espèce en Belgique septentrionale, et propose des pistes d'amélioration de la gestion de ces terres de bruyère pour en favoriser l'implantation.

Introduction

The European Amazon ant, *Polyergus rufescens* (Latreille, 1798) is a critically endangered species in Flanders (DEKONINCK *et al*., 2003; 2012) with a preference for xerophilous dry heath. Due to its specialized behaviour, this dulotic species appeals to everyone's imagination. Equipped with a pair of pointed falcate jaws, workers morphologically indicate that they are not built to perform domestic tasks like foraging and taking care of the offspring. These ants form mixed colonies with species from the subgenus *Serviformica* Forel, 1913 which are kept as slaves and perform all necessary tasks in the nest. The potential of these servant ants is maintained by means of raids organized to collect pupae from neighbouring nests. During raids, organized in the afternoons on hot summer days, they march in columns in search of nests of *Formica fusca* Linnaeus, 1758, *Formica cunicularia* Latreille, 1798 and *Formica rufibarbis* Fabricius, 1793, robbing the pupae and larvae that are reared to eclosion in their own nest. The hatched workers of the servant ants will from now on, be responsible for the maintenance of the mixed colony (SEIFERT, 2018). Observations in an artificial nest construction have shown that *F. rufibarbis* is more aggressive than *F. fusca* and *F. cunicularia*, which can lead to a bias in choosing a nest during a raid (MORI *et al*., 2001).

MATING STRATEGIES

As is the case with other ant species, under favourable conditions ant communities of the Amazon ant can reach the stage in which reproductive gynes and males are produced. Due to the costs, not every population of *P. rufescens* will automatically enter the phase of producing males and females because within the community the decision may be in the first place to expand the strength of the nest by producing more workers (BOROVSKY & BOROVSKY, 2014). After mating, the inseminated queens will be able to start a new nest elsewhere with varying success. There are three main scenarios for reproduction and dissemination. Copulation in mating flights, mating on the ground near the dulotic nest or copulation of gynes participating in the raidings.

Finding a single alate or dealate gyne does not lead automatically to the conclusion that we are dealing with a fertilised gyne searching for a host nest on her own. Observations in the field have led to the hypothesis that after the nuptial flight fertilised females sometimes spend several days in the vicinity of a dulotic nest, not necessarily their own awaiting a raid to which they can participate. However, there are also winged females that do not participate in a nuptial flight but mate on the ground outside their parental nest and attract males through sex pheromones in what is named the female-calling syndrome (MORI *et al*., 2001).

Mating must be followed by finding and entering a host colony of *Serviformica*. After a nuptial flight, fertilized gynes can traditionally fly away in search of a new territory to colonise, hopefully with the presence of the suitable host, but observations show that they are more likely to participate in raidings within their own region. The advantage of participating in a raid is the chaotic situation that arises in a targeted nest during the attack, which makes it easier for the queens to enter. Once the queen of *P. rufescens* has entered the conquered nest, she can release the substances of her Dufour's gland to suppress aggression towards her and encourage its adoption by the *Serviformica*'s workers (Fig. 1).

A RARE SPECIES IN BELGIUM

Jean Bondroit mentions the presence of *Polyergus rufescens* at Yvoir near Dinant (BONDROIT, 1918). In an earlier publication he writes that 'someone with knowledge of entomology' reported to him the observation of a single specimen, one that was not preserved because it was blown away by the wind (BONDROIT, 1911). This makes the first observation questionable.

Fig. 1. Worker of *Polyergus rufescens* returning to the dulotic nest with a captured pupa after a raid on a nest of a *Serviformica* species. © Pavel Krásenský.

This uncertainty is reinforced by the fact that until now *P. rufescens* has not been observed in Wallonia despite intensive research by the 'Groupe de Travail Fourmis Walbru' (WEGNEZ *et al.,* 2012).

The first certain observation in Belgium was made by Jos Van Brabant who found on the 12th of July, 1943 a colony in Diest (RAIGNIER & VAN BOVEN, 1949). Jos Van Brabant has been able to observe this colony for four seasons during which he witnessed several raids aimed at the nests of *Formica fusca*. In 1946 he estimated the number of workers at 1500 and he was also able to determine a number of ergatogynes. On one occasion he witnessed a remarkable colony founding with an initial alliance between a gyne of the Amazon ant and the resident queen of *F. fusca* until the appearance of the first workers of *P. rufescens*. Jozef van Boven mentions in 1963 another observation in Genk, but this colony disappeared due to the construction of the Ford factories (VAN LOON *et al*., 2019).

In 2000 the species was rediscovered by Wouter Dekoninck at the nature reserve the Hageven in Neerpelt (DEKONINCK *et al*., 2001) where the species was last seen in 2004 (VAN LOON *et al*., 2019). This nature reserve forms a larger area in combination with the Plateau on the territory of the Netherlands, where annual monitoring was also carried out in view of the presence of *P. rufescens*. In 2019 the Plateau was intensively investigated with the focus on the area surrounding the old nest locations of the species which in June resulted in the discovery of a colony (NOORDIJK *et al*., 2019; VAN LOON *et al*., 2019). More than 10 years after the last record, Amazon ants were also captured at Balimheide in Lommel (Belgium) in two consecutive years, 2017 (1 worker on 22 September) and 2018 (5 workers on 5 July). Unfortunately, the species will probably disappear here due to the construction of a solar panel park of 100 ha (VAN LOON *et al*., 2019).

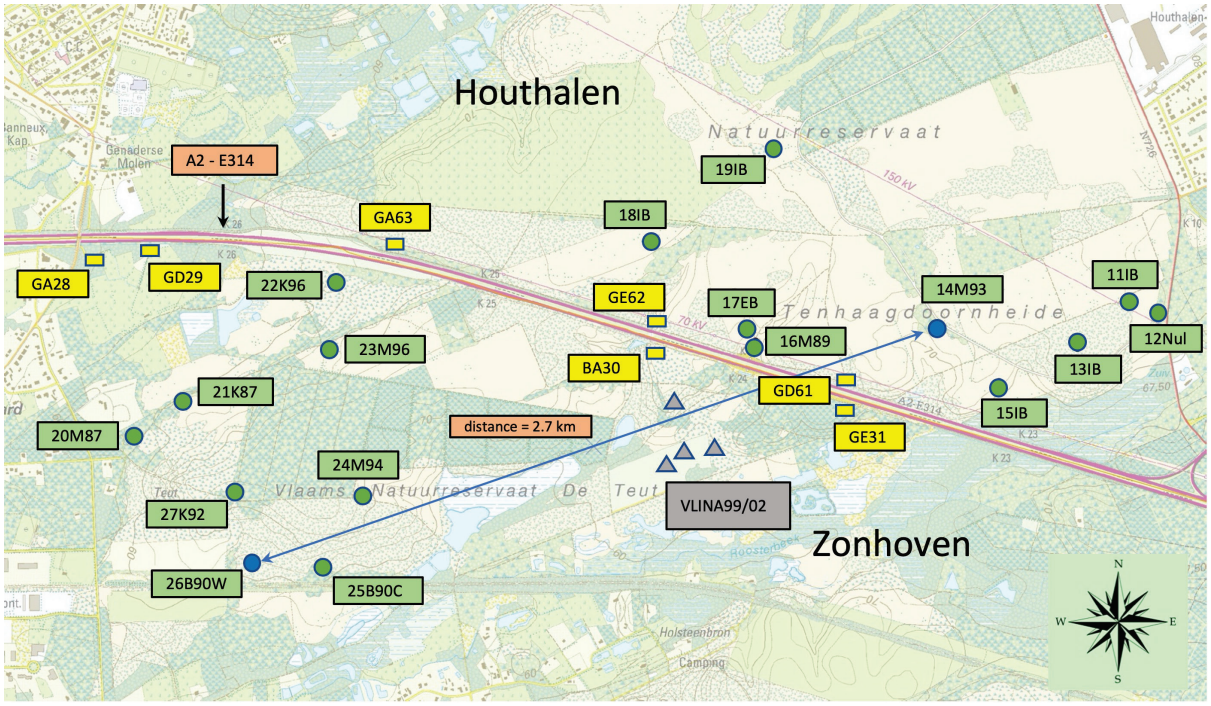

Fig. 2. Map of the investigated heathland. The codes next to the green circles refer to the parallel studies from 1999/2020; the codes of the yellow rectangles indicate the locations of the pitfall traps from the investigation of the road verges along the A2-E314 performed in 1999; the gray triangles labelled VLINA99/02 indicate the locations of the 2000 study. The blue dots (26B90W; 14M93) indicated with a blue arrow represent the locations where the Amazon ants were observed.

From this list of observations, it is clear that in Belgium the Amazon ant is one of the most threatened and rarest observed ant species. Nevertheless, in a large-scale study, where biodiversity of soil invertebrates was investigated in two large heathlands in Zonhoven and, Houthalen several workers and one dealate gyne of the European Amazon ant were collected in Zonhoven, and two dealate and one alate gyne were detected in one plot in Houthalen. In view of the scant observations of *P. rufescens* during the last decades in Belgium and the neighbouring country the Netherlands this observation offers hopeful perspectives for the conservation of this species.

Material and methods

Sampling & Sampling area
(Fig. 2)

The Belgian area under investigation is a 1700 ha heathland, spread over the municipalities of Houthalen and Zonhoven, including Nature reserves De Teut and Tenhaagdoornheide. Originally these nature reserves were a continuous area but in the early 1970's it was bisected by the highway A2-E314.

The study that was set up in 2020 aimed to make a comparative analysis with an earlier survey of the same area in 1999 (Lambrechts *et al.*, 1999a). In 1999, 17 plots were selected in De Teut and Tenhaagdoornheide to collect samples of soil invertebrates using pitfall traps. For this comparison, the same method was applied and the same plots from the earlier study were reinvestigated. From the 17 plots only five contained *Serviformica* ants in the first study of 1999 (Table 1).

A second study (Lambrechts *et al.*, 1999b) looked at soil invertebrates of the road verges along the A2-E314 highway in Limburg. Pitfall traps were placed in 36 locations, 18 plots on

each side of the road between Houthalen/Zonhoven and Maasmechelen. Three plots with three pitfall traps each were singled out on the territory of Houthalen, GD61 (UTM-grid FS7053), GE62 (FS7053), GA63 (FS6954) and four on the side of Zonhoven, GA28 (FS6653), GD29 (FS6754), BA30 (FS7053), GE31 (FS7053). For the subgenus *Serviformica*, only *F. fusca* (traps BA30) was noted for Zonhoven and *F. cunicularia* (traps GE62) and *F. rufibarbis* (traps GA28 & GA63) for Houthalen.

Table 1. Plots with the presence of *Serviformica* ants in previous studies in Zonhoven, Houthalen.

		Formica cunicularia Latreille, 1798	*Formica fusca* Linnaeus, 1758	*Formica rufibarbis* Fabricius, 1793
Study De Teut / Tenhaagdoornheide (1999)	14M93		x	
	17EB			x
	19IB		x	x
	20M87		x	x
	23M96		x	x
Study highway A2-E314 (1999)	GA28			x
	BA30		x	
	GE62	x		
	GA63			x
Study VLINA99/02 (2000)	TEUT14	x	x	x
	TEUT15	x	x	x
	TEUT16	x	x	
	TEUT17	x	x	x

From April 2000 to October 2000, a third study (GROOTAERT *et al*., 2001) was conducted in Nature reserve De Teut. Specimens of invertebrates were sampled in four plots (TEUT14, TEUT15, TEUT16 and TEUT17). In the plots TEUT14, TEUT15 and TEUT17 *F. cunicularia*, *F. fusca* and *F. rufibarbis* were collected; in plot TEUT16 *F. cunicularia* and *F. fusca* were noticed while *F. rufibarbis* was missing (GROOTAERT *et al*., 2001). Neither in the 1999 nor the 2000 studies, Amazon ants were observed.

To evaluate the biodiversity in the 2020 project, the seventeen plots from the previous study by LAMBRECHTS *et al*. (1999a) were selected, nine of them in Tenhaagdoornheide and eight in De Teut. In March 2020 three pitfall traps (labelled A, B and C) were placed in each plot at a distance of five meters. Each jar, with a diameter of 11 cm contained a 2.0% formaldehyde solution, and a roof was installed to cover the trap to prevent rain entering the trap. The traps were emptied with an interval of two weeks (cf. method of LAMBRECHTS *et al*., 1999a).

As specimens of *P. rufescens* were detected in two of the plots examined, plot 14M93 (UTM-grid FS7053) in Houthalen and plot 26B90W (FS6852) in Zonhoven we discuss these plots in detail.

The three pitfall traps of 26B90W in Zonhoven were placed in a small strip of dead heather (*Calluna vulgaris* (L.) Hull) which is part of a larger dry open heather area with some trees on dry sandy soil with a clear iron and/or humus B horizon. A small group of trees with *Pinus sylvestris* L., *Quercus robur* L. and *Prunus serotina* Ehrh. grows directly to the north of these traps. The pitfall traps in this section were surrounded by a monotonous high *C. vulgaris* vegetation. A larger stroke of dead heather also occurs to the southwest of the locality of the pitfall traps with some specimens of *Rumex acetosella* L. and *Deschampsia flexuosa* (L.) Trin. The vegetation type (dry heathland) was in 2020 largely the same as in 1999 (LAMBECHTS *et al*., 1999a). The main difference is that large parts of dead heather were present at these plots in 2020. In grids of 4 square meters around each pitfall traps, the land cover of heather was 43% in 2020 compared to 95% in 1999. In 1990 the open heath area of De Teut was burned

by the manager. Furthermore, the management included mowing, which was done in the immediate vicinity, but not at the specific plot. Finally, most of the sapling trees were also manually removed.

The pitfall traps of plot 14M93 in which gynes of *P. rufescens* were collected at the nature reserve Tenhaagdoornheide were located on the edge of a large open heathland bordered by a row of trees. The open heath area is dominated by *C. vulgaris* with sods of *Molinia caerulea* (L.) Moench in between. Next to the low vegetation *P. sylvestris* L. and *Betula pendula* Roth. grow in the immediate vicinity of the pitfall traps. There is also a concentration of *Q. robur* L. at a greater distance with *Festuca filiformis* Pourr. growing at the tree edge next to *Carex pilulifera* L. and *R. acetosella.* Compared to 1999, there is no remarkable difference to mention other than the distance between the pitfall traps and the trees which is 5 m in the study of 2020 because some new trees have emerged, and 30 m in 1999. The composition of the soil is comparable to De Teut, namely a dry sandy soil with a clear iron and/or humus B layer (Fig.3).

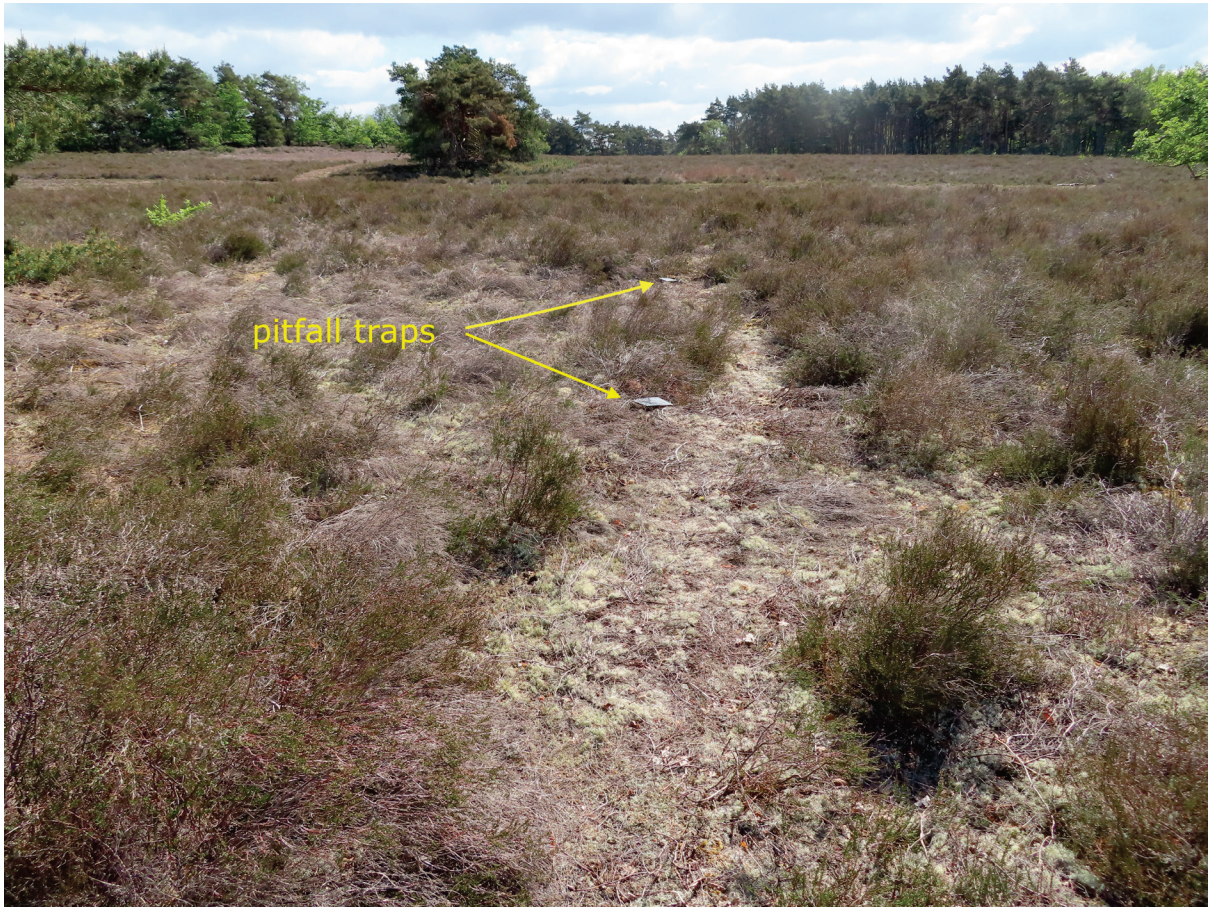

Fig. 3. View on plot 26B90W in De Teut (Zonhoven) with two of the three pitfall traps with a roof to prevent rain entering the traps.

Results and discussion

Observations summer 2020

The observations made in the summer of 2020 confirm the presence of the Amazon ant in De Teut and Tenhaagdoornheide. Furthermore, it gives us the opportunity to discuss its colonization and territorial expansion in that area (Table 2). The presence of a population of *P. rufescens* in De Teut at Zonhoven is abundantly clear from the observation of several workers at different dates in pitfall traps 26B90W-A and 26B90W-B. The capture of a dealate female

together with several workers in plot 26B90W-A in the period 21 July to 4 August might confirm other observations where a queen takes part in a slave-raid leading to a potential host (Table 3). Interpretation of the females present in the reserve Tenhaagdoornheide in Houthalen gives less certainty for the presence of a population. We therefore have two options to explain the presence of these gynes. After a nuptial flight within the population in Zonhoven, the fertilized females have reached the reserve Tenhaagdoornheide and are trying to take over a nest from *Serviformica*. The distance to be bridged is 2.7 km between plot 14M93 and 26B90W and the two reserves are separated by the highway A2-E314 which should not be an unbridgeable obstacle for alate gynes. The second option allows us to assume that there is also a population of the Amazon ant in the Tenhaagdoornheide reserve from which these females originate. The consideration of this second option leaves room to conclude that both reserves have already been colonized by *P. rufescens*.

Table 2. List of ant species present in the plots where *P. rufescens* was noticed. The yellow marked species are gynes, alate or dealate.

	14M93-A	14M93-B	14M93-C	26B90W-A	26B90W-B	26B90W-C
Formica cunicularia Latreille, 1798				x		
Formica fusca Linnaeus, 1758	x			x	x	
Formica fusca Linnaeus, 1758	x					
Formica pratensis Retzius, 1783	x	x	x	x	x	x
Formica rufibarbis Fabricius, 1793			x			
Formica sanguinea Latreille, 1798					x	
Lasius brunneus (Latreille, 1798)				x		
Lasius flavus (Fabricius, 1781)					x	
Lasius fuliginosus (Latreille, 1798)	x			x	x	
Lasius meridionalis (Bondroit, 1920)				x	x	
Lasius platythorax Seifert,1991	x					
Lasius umbratus Nylander, 1846						x
Lasius umbratus Nylander, 1846		x		x		x
Myrmecina graminicola (Latreille, 1802)				x		
Myrmica ruginodis Nylander, 1846		x	x			
Myrmica sabuleti Meinert, 1861	x				x	x
Myrmica sabuleti Meinert, 1861			x			
Myrmica scabrinodis Nylander, 1846		x				
Myrmica schencki Viereck, 1903			x			
Polyergus rufescens (Latreille, 1798)				x	x	
Polyergus rufescens (Latreille, 1798)		x	x	x		
Solenopsis fugax (Latreille, 1798)						x
Strongylognathus testaceus (Schenck, 1852)						x
Temnothorax affinis (Mayr, 1855)						x
Tetramorium caespitum (Linnaeus, 1758)	x	x	x	x	x	
Tetramorium caespitum (Linnaeus, 1758)						x

The results of the investigation do not argue in favour of a large density of *Serviformica* nests nevertheless necessary for long-term vitality of Amazon ant populations as mentioned earlier. *F. cunicularia* is missing in Tenhaagdoornheide (14M93-A, 14M93-B & 14M93-C) while *F. rufibarbis* isn't found in De Teut (26B90W-A, 26B90W-B & 26B90W-C). Moreover, only a few specimens of the species in question were collected. In contrast to the minute presence of *Serviformica, F. pratensis* is dominant in the plots in the reserves Tenhaagdoornheide (14M93) and De Teut (26B90W).

To stop collecting specimens of *P. rufescens*, the pitfall traps at 26B90W were removed the 21st of August. On the 14th of September we visited plot 26B90W in the afternoon hoping to witness raiding activities. It was a sunny, windless day with a temperature of 30 degrees Celsius. A single Amazon ant was observed on a rotten trunk of a birch lying between some

pines growing on the edge of the open area, separated from the collection site by a dense heath. Further investigation around and under this tree trunk yielded no further specimens and showed no indication of a nest present. Only a few *F. fusca* ants were foraging under this trunk.

The peak of the observed activities is manifested in the period from 21 July to 4 August. Here we found two dealate and one alate gyne scattered over three locations. We are considering the possibility that there may have been two raids, either on the same day or on two consecutive days.

Table 3. Number of the sampled specimens of *P. rufescens* by location and date. The date displayed is the end date of a fortnight period.

	Tenhaagdoornheide			**De Teut**		
	Houthalen			**Zonhoven**		
	14M93-B	14M93-C		26B90W-A		26B90W-B
	dealate gyne	alate gyne	dealate gyne	dealate gyne	worker	worker
9 June						1
23 June					4	1
7 July					12	
4 August	1	1		1	44	148
18 August					5	9
15 September			1			

This research does not allow us to determine with certainty which strategy was followed. Additionally, the exact date of the raids or nuptial flights can only be deduced by analogy of other field observations. In order to arrive at a probability bordering on certainty, we consider the climatic observations for this period. This leads us to the most plausible conclusion that three dates are most likely, namely 30 July, 31 July and 1 August, days characterized by a dry, sunny and warm period with maximum temperatures between 28.2° Celsius and 35.6° Celsius (Table 4).

A remarkable phenomenon for the Amazon ant is the time difference between a nuptial flight and a raid. Mating flights were mostly observed in the early afternoon (around 14.00h) while raiding activity mainly took place in the late afternoon (16.00h–18.00h) (SEIFERT, 2018). This observation does not automatically lead to the conclusion that this is a planned strategy, although it is in line with the fact that females regularly join the raids and are simultaneously escorted to a potential host nest where they can establish a new colony.

Table 4. Overview of the weather conditions during the period under review. Three consecutive days with optimal conditions for raiding activity are highlighted in yellow.

	21 July	**22 July**	**23 July**	**24 July**	**25 July**	**26 July**	**27 July**	**28 July**	**29 July**	**30 July**	**31 July**	**1 August**	**2 August**	**3 August**	**4 August**
average T (° C)	16.5	15.7	18.7	18.9	19.7	19.1	19.9	19.5	17.8	19.6	23.3	24.6	17.7	15.3	16.5
maximum T (° C)	23.5	23.7	26.4	23.1	24.5	23.1	29.3	23.2	24.0	28.2	35.6	28.9	22.8	24.6	24.3
humidity (%)	63.7	62.3	58.3	69.6	73.9	75.6	62.7	63.7	60.2	57.1	30.1	59.5	56.6	41.1	35.3
wind speed (km/h)	5.8	7.4	8.2	10.5	13.0	14.8	12.1	16.1	10.5	7.7	11.3	10.8	9.5	7.4	12.1
atmospheric pressure (hPa)	1,015.5	1,014.9	1,009.2	1,004.3	1,002.9	1,002.6	1,003.7	1,003.8	1,011.0	1,011.4	1,006.9	1,005.4	1,006.8	1,005.4	1,007.5
precipitation (mm)	19	0	0	0	0.6	1.8	0	0	0	0	0	0	0	0	0

Conclusions

PRESENT BUT VULNERABLE

The results lead to the conclusion that a colony of *P. rufescens* is certainly present in De Teut. We can only suspect this for Tenhaagdoornheide, but the observation of gynes is hopeful with regard to colonization. The crucial point for the preservation and expansion of the Amazon ant is the presence of *Serviformica* ants. In the habitat of *P. rufescens* it is necessary to maintain the balance between hosts and slaves. This equilibrium can be very fragile because several factors can create a negative impact. Reduced food supply as well as drastic changes in the biotope can affect the population density of the host. An excessive increase in the population of the Amazon ant itself could threaten their own survival. Every queen of *P. rufescens* who manages to take over a nest of a *Serviformica* species automatically contributes to the reduced supply of the host species. A too low supply of *Serviformica* larvae and pupae within the habitat can also cause the Amazon ant to switch to intraspecific raids with elimination of a colony (MORI *et al*., 1994).

The obligatory parasitic ant *P. rufescens* is not that easy to detect, especially in a region where the populations are rather small. Due to their dulotic nature, finding a nest is difficult because the workers of the Amazon ant are never found dwelling around the nest unless during the summer raids. Throughout the year all tasks are performed by the slaves such as foraging and defending the nest. Therefore, predominantly the servant ants will be observed outside the nest and without the outdoor activity of Amazon ants there is no indication for the presence of a dulotic colony. To witness a raid, the observer must be in the right place at the right time (DEKONINCK & VANKERKHOVEN, 2001; DEKONINCK *et al*., 2012). As a consequence, the presence of *P. rufescens* was mainly detected in Belgium during long-term studies by using pitfall traps.

Once the presence of the Amazon ant is known, its evolution can be monitored by visiting the site on a regular basis taking into account the time of year coupled with the right weather conditions. Despite having some idea where *P. rufescens* colonies can be found, this remains a labour-intensive undertaking. After years without observations, the researchers in the Netherlands wondered whether the Amazon ant had become extinct. After a two-year study, the conclusion was drawn that the Amazon ant was extinct in the Netherlands until, during a field visit in 2019, workers were found purely by chance (MABELIS, 2000; VAN LOON *et al*., 2019).

Dry sandy heathland seems to be a preferred habitat for *P. rufescens,* but the habitat must also meet the requirements of the servant ants. Trees and bushes in the vicinity of dulotic nests can contribute to the food supply for *Serviformica* ants by harbouring aphids, visited by these ants for their sweet secretions.

Hot and dry periods during summertime, frequently noticed in recent years can contribute to the requirements preferred by the more thermophilic Amazon ants. The population recently found in Zonhoven and Houthalen seems to be a promising indication for settlement and dispersion in Belgium. Their presence is an encouragement to thoughtful management of the habitat of a special ant that deserves our protection.

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